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Short communication

DNA Barcoding of the Endangered Species *Ellobium chinense* (Mollusca, Gastropoda, Ellobiidae) from Coastal Areas of South Korea

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ABSTRACT

The pulmonate gastropod *Ellobium chinense* (Pfeiffer, 1864) is an endangered marine species along the South Korean coasts due to habitat destruction and population declines. We sequenced the cytochrome *c* oxidase subunit 1 (COI) of 25 *E. chinense* specimens collected from five coastal sites in South Korea, and identified 16 unique haplotypes. The maximum intraspecific variation among individuals was 1.6%, while interspecific differences from another ellobiid species, *Auriculastra duplicata* (Pfeiffer, 1854), ranged from 21.9 to 23.0%. Our barcoding data will be useful to elucidate the phylogenetic relationships among pulmonate gastropods and infer the population genetic structure of *E. chinense*.

Keywords: DNA barcode, cytochrome *c* oxidase subunit I, marine endangered species, pulmonate

INTRODUCTION

Ellobium chinense (Pfeiffer, 1864) is a conoidal pulmonate gastropod mollusk (Fig. 1) that inhabits the intertidal and terrestrial zones of estuaries and coastal regions. This species' whole distribution range is known to be confined to the northwestern Pacific coasts of Asia, including China, Japan, and South Korea (Yoo, 1976). In South Korea, it mostly inhabits saltmarshes along the western and southern coasts, where it has been severely affected by human activities, such as land reclamation and coastal development, during the last few decades (Lim et al., 2015). Therefore, this species is regarded as a being in need of conservation in South Korea, and has been registered as an endangered species by the Korean Government (Ministry of Environment, 2016; Ministry of Oceans and Fisheries, 2017). Although genetic resources of endangered species are essential for the success of conservation strategies concerned with the preservation of their populations and habitat restoration, only limited genetic data have been published in public databases on them until recently. In this study, we reported the cytochrome *c* oxidase subunit 1 (COI) sequences of *E. chinense* specimens collected from five coastal areas of South Korea. Additionally, we analyzed genetic distances among *E. chinense* individuals, and compared with an ellobiid species *Auriculastra duplicata* (Pfeiffer, 1854) (Gastropoda, Ellobiidae), which exhibits similar ecological and morphological characteristics to those of *E. chinense* (see Lee and Lee, 2015; Yi et al., 2017). Unfortunately, there was no compatible sequences of *Ellobium* species in public databases.

[Permission for sampling, analysis, and storage of *E. chinense* was obtained from the Geum River Basin Environmental Office (Permit No. 2015-14), Saemangeum Regional Environmental Office (Permit No. 2015-09), Yeongsan River Basin Environmental Office (Permit No. 2015-21), and Nakdong River Basin Environmental Office (Permit No. 2015-18), as this species is protected as a type of endangered wildlife by law.]

RESULTS AND DISCUSSION

The COI sequences of *E. chinense* were obtained from five coastal areas in South Korea: Seocheon (36°8′23″N, 126°34′16″E), Gochang (35°31′50″N, 126°35′47″E), Haenam (34°24′41″N, 126°38′3″E), Yeosu (34°41′42″N, 127°34′49″E), and

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Fig. 1. Photographs of *Ellobium chinense* from Sacheon. A, Dorsal; B, Ventral (photo taken in July 2015).

Sacheon (35°2′57″N, 128°0′31″E). In total, 25 adult (4–6 cm in shell length) specimens were collected between March and August of 2015, with five individuals collected from each of the sites. Morphological identification was performed based on the description of this species given by Yoo (1976), and all specimens were deposited in the National Marine Biodiversity Institute of Korea (MABIK) (Seocheon, Korea). Voucher numbers are given in Table 1. From the extracted genomic DNA, COI sequences were amplified with the following newly designed two-primer sets: Mmt00002f (5′-TGC GTT GGY TAT TYT CMA CAA A-3′) and Mmt00811r (5′-ATC CCA ATY GAW ACT ATG GC-3′), and Mmt00019f (5′-ACA AAY CAY AAA GAT ATT GG-3′) and Mmt00826r (5′-ACA ATA AAM CCY AAA ATY CC-3′). Amplified sequences were then aligned using Geneious 9.1.8 (Biomatters Ltd., Auckland, New Zealand). Newly obtained 631 bp COI sequences were registered in the GenBank nucleotide database (accession Nos. MK696944–696968). Pairwise genetic distances among sequences were calculated in the MEGA X program (Kumar et al., 2018) using the Kimura two-parameter model (Kimura, 1980). The COI sequences of *A. duplicata* (NC036959) were compared as outgroups to those of *E. chinense*. In our results,

Table 1. Pairwise genetic distances among individuals, with their collecting locations, NCBI accession numbers, and specimen voucher numbers

Species	Location	No.	NCBI No.	Voucher No.	Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Ellobium chinense	Seocheon	1	MK696944	MO00163264	A																									
		2	MK696945	MO00163265	B	0.013																								
		3	MK696946	MO00163266	C	0.010	0.013																							
		4	MK696947	MO00163267	D	0.010	0.016	0.010																						
		5	MK696948	MO00163268	E	0.010	0.003	0.010	0.013																					
	Gochang	6	MK696949	MO00163113	F	0.011	0.014	0.005	0.011	0.011																				
		7	MK696950	MO00163114	G	0.006	0.010	0.010	0.010	0.006	0.011																			
		8	MK696951	MO00163115	H	0.005	0.011	0.008	0.008	0.008	0.010	0.005																		
		9	MK696952	MO00163116	G	0.006	0.010	0.010	0.010	0.006	0.011	**0.000**	0.005																	
		10	MK696953	MO00163117	G	0.006	0.010	0.010	0.010	0.006	0.011	**0.000**	0.005	**0.000**																
	Haenam	11	MK696954	MO00163230	I	0.010	0.013	0.013	0.013	0.010	0.014	0.010	0.008	0.010	0.010															
		12	MK696955	MO00163231	J	0.003	0.010	0.006	0.010	0.006	0.008	0.006	0.005	0.006	0.006	0.010														
		13	MK696956	MO00163232	K	0.008	0.011	0.008	0.011	0.008	0.010	0.008	0.006	0.008	0.008	0.008	0.005													
		14	MK696957	MO00163233	I	0.010	0.013	0.013	0.013	0.010	0.014	0.010	0.008	0.010	0.010	**0.000**	0.010	0.008												
		15	MK696958	MO00163234	L	0.010	0.016	0.013	0.003	0.013	0.014	0.010	0.008	0.010	0.010	0.013	0.010	0.011	0.013											
	Yeosu	16	MK696959	MO00163157	E	0.010	0.003	0.010	0.013	**0.000**	0.011	0.006	0.008	0.006	0.006	0.010	0.006	0.008	0.010	0.013										
		17	MK696960	MO00163158	M	0.008	0.014	0.011	0.011	0.011	0.013	0.008	0.006	0.008	0.008	0.011	0.008	0.010	0.011	0.011	0.011									
		18	MK696961	MO00163159	N	0.005	0.011	0.008	0.008	0.008	0.010	0.002	0.003	0.002	0.002	0.008	0.005	0.006	0.008	0.008	0.008	0.006								
		19	MK696962	MO00163160	M	0.008	0.014	0.011	0.011	0.011	0.013	0.008	0.006	0.008	0.008	0.011	0.008	0.010	0.011	0.011	0.011	**0.000**	0.006							
		20	MK696963	MO00163161	I	0.010	0.013	0.013	0.013	0.010	0.014	0.010	0.008	0.010	0.010	**0.000**	0.010	0.008	**0.000**	0.013	0.010	0.011	0.008	0.011						
	Sacheon	21	MK696964	MO00163118	O	0.008	0.014	0.011	0.002	0.011	0.013	0.008	0.006	0.008	0.008	0.011	0.008	0.010	0.011	0.002	0.011	0.010	0.006	0.010	0.011					
		22	MK696965	MO00163119	C	0.010	0.013	**0.000**	0.010	0.010	0.005	0.010	0.008	0.010	0.010	0.013	0.006	0.008	0.013	0.013	0.010	0.011	0.008	0.011	0.013	0.011				
		23	MK696966	MO00163120	P	0.005	0.011	0.008	0.008	0.008	0.010	0.005	0.003	0.005	0.005	0.005	0.005	0.006	0.005	0.008	0.008	0.006	0.003	0.006	0.005	0.006	0.008			
		24	MK696967	MO00163121	E	0.010	0.003	0.010	0.013	**0.000**	0.011	0.006	0.008	0.006	0.006	0.010	0.006	0.008	0.010	0.013	**0.000**	0.011	0.008	0.011	0.010	0.011	0.010	0.008		
		25	MK696968	MO00163122	E	0.010	0.003	0.010	0.013	**0.000**	0.011	0.006	0.008	0.006	0.006	0.010	0.006	0.008	0.010	0.013	**0.000**	0.011	0.008	0.011	0.010	0.011	0.010	0.008	**0.000**	
Auriculastra duplicata	Ganghwa	26	NC036959	MO00163733	–	0.223	0.225	0.221	0.230	0.225	0.223	0.230	0.223	0.230	0.230	0.221	0.221	0.221	0.221	0.230	0.225	0.219	0.228	0.219	0.221	0.228	0.221	0.228	0.225	0.225

Specimens were deposited in National Marine Biodiversity Institute of Korea (MABIK). Bold numbers indicate no difference between specimens.

a total 16 haplotypes of the mtDNA COI sequences were identified from the 25 individuals examined. Among the five sampling sites, only specimens from Gochang did not share any haplotypes with specimens from any other sites (Table 1). The maximum intraspecific genetic variation was 1.6% among all *E. chinense* specimens, while the interspecific genetic difference of *E. chinense* from *A. duplicata* ranged from 21.9 to 23.0%. In conclusion, the use of the mtDNA COI region of *E. chinense* was found to be appropriate for identifying this species and its related taxon due to their low intraspecific genetic variations and high interspecific variations in this gene. In addition, the relatively high haplotype diversity of this region will make it possible to analyze population genetic diversity and structure to help in establishing conservation strategies for *E. chinense*.

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REFERENCES

Kimura M, 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16:111-120. https://doi.org/10.1007/BF01731581

Kumar S, Stecher G, Li M, Knyaz C, Tamura K, 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution, 35:1547-1549. https://doi.org/10.1093/molbev/msy096

Lee JS, Lee YS, 2015. A report of three unrecorded Ellobiid species (Gastropoda, Eupulmonata) from Korea. Korean Journal of Malacology, 31:323-326. https://doi.org/10.9710/kjm.2015.31.4.323

Lim SY, Lee CS, Kim MS, Yoo SH, 2015. The conservation value of endangered marine species: the case of the *Ellobium chinense*. Journal of the Korean Society of Marine Environment & Safety, 21:645-654. https://doi.org/10.7837/kosomes.2015.21.6.645

Ministry of Environment, 2016. Wildlife Protection and Management Act [Internet]. Korea Ministry of Government Legislation, Sejong, Accessed 10 Apr 2019, <http://www.law.go.kr/lsInfoP.do?lsiSeq=180446&urlMode=engLsInfoR&viewCls=engLsInfoR#0000>.

Ministry of Oceans and Fisheries, 2017. Conservation and Management of Marine ecosystems Act [Internet]. Korea Ministry of Government Legislation, Sejong, Accessed 10 Apr 2019, <http://www.law.go.kr/LSW/eng/engLsSc.do?menuId=2&query=CONSERVATION%20AND%20MANAGEMENT%20OF%20MARINE%20ECOSYSTEMS%20ACT>.

Yi CH, Kim KY, Jung TW, Cho IY, Kim IH, Hong SS, Hwang SJ, Yoon M, Kim W, Han D, Kim MS, 2017. Complete sequence analysis of the mitochondrial genome of *Auriculastra duplicata* (Mollusca, Gastropoda, Ellobiidae). Mitochondrial DNA Part B, 2:787-788. https://doi.org/10.1080/23802359.2017.1398614

Yoo JS, 1976. Korean shells in color. Il Ji Sa Publishing Co., Seoul, pp. 1-196.

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